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Multiple Antimicrobial Interventions for the Control of Escherichia coli O157:H7 in Very Small Beef Processing Facilities

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Summary

One-hundred and fifty beef carcasses from 3 very small beef processing plants were sponge sampled for aerobic plate count, generic E. coli, *coliforms,* Enterobacteriaceae, *and* E. coli *O157:H7 before and after carcass intervention strategies. The control (C) treatment consisted of one 3% lactic acid (LA) wash applied at the end of slaughter, just prior to chilling. The multiple (M) intervention treatment received a 3% LA wash prior to evisceration, a hot water wash after carcass splitting and trimming, and a final LA wash just prior to chilling. The M treatment showed greater log reductions throughout the slaughter process prior to chilling for indicator bacteria. M and C treatments were similar for all bacteria after chilling. Both treatments were effective at reducing the occurrence of* E. coli *O157:H7.*

Introduction

Beef processing plants of all sizes have implemented intervention technologies throughout the slaughter process to reduce or eliminate microorganisms. Published research has shown several different antimicrobial agents used as a carcass spray intervention to be effective at reducing a variety of bacteria and pathogens. Many antimicrobial agents involve the use of organic acids and/or heat as interventions, with lactic acid, acetic acid, and hot water being the most common antimicrobial interventions.

Antimicrobial interventions can be used alone or in conjunction with additional interventions throughout the slaughter process and are commonly referred to as multiple intervention systems. The use of multiple interventions has been effective at reducing bacterial contamination in a laboratory and large commercial beef processing facilities. However, little research is available on the effectiveness of multiple interventions in small or very small beef processing facilities, which comprise about 83% of the federally inspected processing plants in Nebraska. Therefore, the purpose of this study was to compare the effectiveness of multiple versus single antimicrobial interventions for the reduction of *E. coli* O157:H7 and other indicator bacteria during the slaughter process in small and very small meat processing facilities.

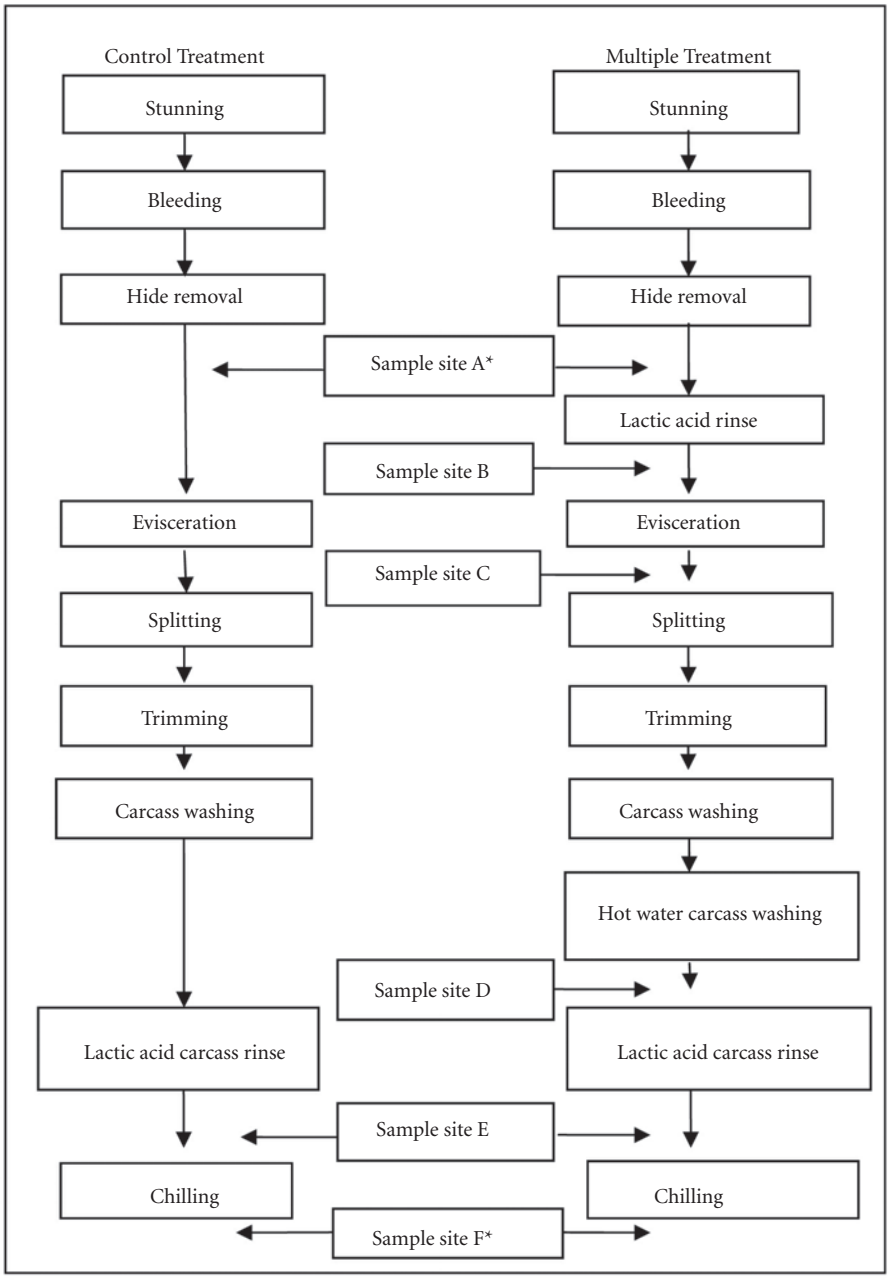
Procedure

Experimental Design

A very small processing plant is defined under the final rule as having fewer than 10 employees or less than $2.5 million in annual sales. One-hundred and fifty beef carcasses were sampled across three very small processing plants for aerobic plate count (APC), coliforms (CL), generic *E. coli* (EC), *Enterobacteriaceae* (EB), and *E. coli* O157:H7. The control (C) treatment (75 carcasses) consisted of a single antimicrobial intervention whereby a 3.0% (vol/vol) lactic acid (LA) spray (≥ 132°F) was applied to the carcass at the end of the slaughter process prior to carcass chilling. The multiple (M) intervention treatment (75 carcasses) consisted of three antimicrobial interventions during the slaughter process: 1) 3.0% (vol/vol) LA spray (≥ 132°F) was applied to the carcass immediately after hide removal and prior to evisceration; 2) hot water intervention (≥ 165°F) was applied after the final carcass wash at the end of the slaughter process; and 3) an additional 3.0% (vol/vol) LA spray (≥ 132°F) was applied to the carcass at the end of the slaughter process just prior to carcass chilling. Chilling rates were recorded on randomly selected carcasses during the 24-hour post-slaughter chilling process.

Hot Water Application

The M intervention carcasses received a 2-minute hot water wash per side. A tankless portable water heater (Rinnai; Nagoya, Japan) with a side mount temperature gauge was utilized to heat water to ≥165°F at carcass surface contact. An in-line water pressure gauge (Span Pressure Gauges; Waukesha, Wisc.) was inserted to measure water pressure at 45-75 psi. An in-line temperature gauge (Trend, Division of WIKA, Lawrenceville, Ga.) also was inserted where the hose and spray gun connect to measure water temperature at the end of the hose. The tip of the spray nozzle (McMaster-Carr, Chicago, Ill.; 50° angle, brass, flat fan spray) was ≤ 12 in from the carcass during hot water application to minimize heat loss. A thermocouple temperature gauge was used to measure water temperature flowing out of the spray nozzle. The temperature gauge was held 12 in from the spray nozzle and temperatures were recorded prior to carcass application. Temperatures were recorded at this distance from the spray nozzle to simulate the water temperature at carcass contact. The tankless water heater was programmed at 185°F to ensure final water temperature ≥ 165°F for carcass application.



**E. coli* O157:H7 sampling locations

Sample site A: after hide removal prior to LA spray and evisceration; B) post LA spray prior to evisceration; C) post evisceration before hot water intervention; D) post hot water intervention; E) post final LA spray, and F) after chilling overnight

Figure 1. Location of antimicrobial interventions and indicator organism sampling sites in the beef slaughter process.

Lactic Acid Application

All carcasses received at least one LA spray for 1 minute per side per application. A 3% (vol/vol) concentration of LA (Birko, Denver, Colo.; Purac America, Linconshire, Ill.; 88% food grade LA) was sprayed on the hot carcasses for both treatments. A stainless steel garden pump sprayer was modified with an air compressor adaptor (NIBCO®; Elkhart, Ind.) to achieve spraying pressure between 20-35 psi. A 1 gallon air compressor (Campbell Hausfeld®; Harrison, Ohio) was used to pressurize the LA spray system. A pressure gauge was mounted in the tank line to record and monitor pressure. The LA solution had a target temperature above 131°F with an acceptable range between 130-140°F. Temperature was measured by a thermocouple temperature gauge prior to carcass application.

Carcass Sampling

Sampling locations were determined on the basis of where the hide was removed from the carcass and probable contamination sites. APC, CL, EC, and EB sponge samples were taken along the navel/plate/midline, brisket, and a portion of the outside round, totaling 100 cm² at each location and 300 cm² per swab. *E. coli* O157:H7 sampling locations were the foreshank, inside round, and the inside portion of the hindshank, as suggested by previous research. The location of antimicrobial interventions and microbiological sampling sites in the beef slaughter process for both treatments are shown in Figure 1. The C treatment was sampled on both sides of the carcass prior to evisceration, post LA spray prior to chilling, and after overnight chilling for indicator organisms.

Sample collection for the M intervention treatment was performed: A) after hide removal prior to LA spray and evisceration; B) post LA spray prior to evisceration; C) post evisceration before hot water intervention; D) post hot water intervention; E) post final LA spray; and F) after chilling overnight. Because of space restrictions on the carcass, the first three sampling sites (A, B, C) were sampled on one side of the carcass, and the last three sampling sites (D, E, F) were sampled on the corresponding side of the same carcass later in the slaughter process to eliminate the possibility of sampling the same area on the carcass. This sampling scheme rotated from side to side on every carcass in the M intervention treatment.

(Continued on next page)

Table 1. LS means for Aerobic Plate Count, *Enterobacteriaceae*, coliforms, and *E. coli* populations (log CFU/cm²) at each sampling site and treatment across all plants.

	Aerobic Plate Count			*Enterobacteriaceae*			Coliforms			*E. coli*		
Sampling site[1]	Control	Multiple	SEM	Control	Multiple	SEM	Control	Multiple	SEM	Control	Multiple	SEM
A	3.17^{w}	2.97^{w}	0.139	1.11^{w}	1.07^{w}	0.134	0.79^{w}	0.83^{w}	0.144	-0.70^{w}	-0.54^{w}	0.101
B	—	2.19^{xy}	—	—	0.43^{x}	—	—	-0.03^{x}	—	—	-1.11^{xy}	—
C	—	2.45^{x}	—	—	0.61^{x}	—	—	0.16^{x}	—	—	-1.00^{xy}	—
D	—	2.45^{x}	—	—	0.61^{x}	—	—	0.07^{x}	—	—	-0.95^{x}	—
E	2.26^{ax}	1.54^{bz}	0.169	0.51^{ax}	-0.01^{by}	0.134	0.00^{ax}	-0.39^{by}	0.142	-1.03^{ax}	-1.19^{by}	0.067
F	2.05^{x}	1.92^{yz}	0.179	0.31^{x}	0.42^{x}	0.149	-0.04^{x}	0.02^{x}	0.151	-1.18^{y}	-1.07^{xy}	0.065

[1]A: log counts post hide removal, pre-evisceration, pre-lactic acid (LA).
B: log counts pre-evisceration, post LA.
C: log counts post evisceration, pre-hot water (HW).
D: log counts post evisceration, post HW, pre LA.
E: log counts post evisceration, post HW, post LA, pre-chill.
F: log counts post evisceration, post HW, post LA, post-chill.
[ab]means within row of common bacteria with differing superscripts differ $P \leq 0.05$.
[wxyz]means within column with differing superscripts differ $P \leq 0.05$.
SEM: standard error of the mean.

Analyses

Samples were shipped in coolers with ice packs to the food microbiology laboratory at the University of Nebraska for microbial analysis. Microbial data for APC, EC, CL, and EB were determined by plating 1 ml of diluted sample homogenate onto 1 of 3 types of Petrifilm™ (3M, St. Paul, Minn.): APC, *E. coli*, coliforms, and ENT (*Enterobacteriaceae*). Petrifilms™ were allowed to dry and then incubated for 48 hours at 95°F before counting. Colonies were reported as colony forming units per square centimeter (CFU/cm²). For the *E. coli*/Coliforms Petrifilm™, blue/purplish colonies with gas production were classified as *E. coli* and all remaining colonies as coliforms. Samples being analyzed for *E. coli* O157:H7 were tested by the USDA-accepted BAX® system PCR assay. An analysis of variance (ANOVA) using the MIXED procedure of SAS was performed for data analyses.

Table 2. LS means for Aerobic Plate Count populations (log CFU/cm²) for combined treatment by plant and sampling sites.

Sampling site [1]	Plant 1	Plant 2	Plant 3	Pr > F
A	2.96^{a}	3.13^{a}	3.11^{a}	0.63
E	1.74^{a}	1.28^{a}	2.68^{b}	< 0.01
F	1.61^{a}	1.51^{a}	2.85^{b}	< 0.01

[ab]differing superscripts between plants at same sampling site differ $P \leq 0.05$.
[1]A: log counts post hide removal, pre-evisceration, pre-lactic acid (LA).
E: log counts post evisceration, post hot water, post LA, and pre-chill.
F: log counts post evisceration, post hot water, post LA, and post chill.

Results

Across all plants, LS means expressed as log counts (CFU/cm²) for APC, EC, CL, and EB were similar ($P \geq 0.15$) for C and M intervention carcasses before interventions were applied (Table 1). The APC, EC, CL, and EB populations for the M intervention carcasses were less ($P \leq 0.03$) than C carcasses after evisceration, hot water, and LA and just prior to carcass chilling. However, treatments were similar ($P > 0.16$) for APC, EC, CL, and EB after chilling (Table 1). Table 2 shows the effect of plant on APC log counts (CFU/cm²) sampled

Table 3. LS means for Aerobic Plate Count, *Enterobacteriaceae*, coliforms, and *E. coli* reductions (log CFU/cm²) at each sampling site and treatment across all plants.

	Aerobic Plate Count			*Enterobacteriaceae*			Coliforms			*E. coli*		
Sampling site[1]	Control	Multiple	SEM	Control	Multiple	SEM	Control	Multiple	SEM	Control	Multiple	SEM
A – B	—	0.77	—	—	0.64	—	—	0.87	—	—	0.57	—
A – C	—	0.51	—	—	0.46	—	—	0.67	—	—	0.46	—
A – D	—	0.52	—	—	0.46	—	—	0.76	—	—	0.41	—
A – E	0.91^{a}	1.42^{b}	0.280	0.59^{a}	1.08^{b}	0.173	0.79^{a}	1.23^{b}	0.184	0.32^{a}	0.65^{b}	0.104
A – F	1.11	1.04	0.218	0.80	0.64	0.189	0.83	0.81	0.210	0.47	0.53	0.102

[1]A – B: log reduction from (post hide removal, pre-evisceration, pre-lactic acid (LA)) to (pre-evisceration, post LA).
A – C: log reduction from (pre-evisceration, pre-LA) to (post evisceration, pre-hot water (HW)).
A – D: log reduction from (pre-evisceration, pre-LA) to (post evisceration, post HW).
A – E: log reduction from (pre-evisceration, pre-LA) to (post evisceration, post HW, post LA, pre-chill).
A – F: log reduction from (pre-evisceration, pre-LA) to (post evisceration, post HW, post LA, post chill).
[ab]means with differing superscripts within similar bacteria log reduction columns differ $P \leq 0.05$.
SEM: standard error of the mean.

Table 4. LS means for reductions (log CFU/cm²) of Aerobic Plate Count by plant and sampling site.

	Plant 1		Plant 2		Plant 3		
Sampling site[2]	Control	Multiple	Control	Multiple	Control	Multiple	Pr > F[1]
A – E	0.68[a]	1.75[b]	1.42[a]	2.26[b]	0.62[a]	0.25[a]	0.02

[ab]means within plant with differing superscripts differ $P < 0.05$.
[1]F-test statistic for the difference of log reduction across plants and treatments.
[2]A - E: log reduction from sampling sites: (post hide removal, pre-evisceration, pre-lactic acid) - (post lactic acid, pre-chill).

Table 5. Number and percentage of *E. coli* O157:H7 positive samples by treatment across all plants.

	Control	Multiple
Sample site A[1]		
Total positives	13[a]	14[a]
Total head sampled	75	75
Total percentage	17.33%	18.66%
Sample site F[2]		
Total positives	2[b]	1[b]
Total head sampled	75	75
Total percentage	2.67%	1.33%

[a,b]differing superscripts within row and column differ $P \leq 0.05$.
[1]Sample site A= after hide removal, before evisceration and interventions.
[2]Sample site F= after all interventions and after 24 hours of carcass chilling.

throughout the slaughter process (sample sites A, E, and F). Plant 3 showed greater ($P < 0.01$) APC populations at sampling sites E and F compared to plants 1 and 2. These data, along with our observation of slaughter operations, suggest plant 3 could standardize sanitary carcass dressing procedures and improve sanitation of skinning knives during slaughter. Similar intervention strategies have been used to reduce log (CFU/cm²) mean values for APC, CL, and EC, including a hot carcass wash (160-170°F) and organic acid sprays (1.6-2.6%; 109-140°F lactic or acetic acid), but in a large commercial setting.

The M intervention carcasses had a greater log reduction ($P = 0.02$) than the C carcasses (1.42 and 0.91 log CFU/cm², respectively) for APC throughout the harvesting process from pre-evisceration until just prior to carcass chilling across all plants (Table 3). EC, CL, and EB also showed greater log reductions ($P = 0.03$) in the M intervention treatment prior to chilling. Similar log reductions ($P = 0.48$) for EC, CL, and EB on carcasses were observed after chilling; however, both treatments achieved greater than one log reduction (CFU/cm²) for APC post chill (Table 3). Table 4 shows reductions (log CFU/cm²) in APC on a plant by treatment basis, where an interaction is noticed. Plants 1 and 2 achieved greater reductions (log CFU/cm²) for the M treatment versus the C treatment throughout the slaughter process and prior to carcass chilling (sampling site A-E). However, plant 3 carcass samples did not show a difference in APC reductions (log CFU/cm²) between the two treatments.

Across all plants (Table 1), the M intervention carcasses, when compared to the C carcasses, experienced a numerical log (CFU/cm²) increase for APC from just prior to chilling (site E) to 24 hr post chill (site F). The reason for this is uncertain; however, it is possible the M intervention carcasses may have experienced more drip loss from the additional four minute hot water wash, and in turn, diluted the concentration of the subsequent LA spray. The hot water wash may have allowed the M intervention carcasses to enter the cooler at warmer temperatures and taken longer to chill; however, temperatures between the treatments were the same. A numerical increase in log counts (CFU/cm²) for APC, EB, and CL was seen after the evisceration step (Sampling site C). Previous research has reported similar findings by using a LA rinse before evisceration and recording a slight increase overall for APC and EB after evisceration, prior to additional interventions and chilling.

Of the 27 positive *E. coli* O157:H7 samples found prior to interventions, 13 (17.3%) and 14 (18.6%) of the positive samples received the C and M intervention treatments, respectively, which were similar ($P = 1.00$) (Table 5). Two carcass samples (2.67%) receiving the C treatment tested positive for *E. coli* O157:H7 after chilling, and one sample (1.33%) in the M intervention treatment tested positive for *E. coli* O157:H7 after chilling. All three post-chill *E. coli* O157:H7 positive samples occurred on the same day at plant 3. Carcasses testing positive for *E. coli* O157:H7 after chilling were treated with a 5% LA solution and re-tested. All re-tested carcasses were negative for *E. coli* O157:H7. Treatments were similar (P=0.69) after the chilling process for positive *E. coli* O157:H7 samples. Both treatments were effective at reducing the occurrence of *E. coli* O157:H7 after interventions were applied.

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